Differential regulation of Rho GTPases by β1 and β3 integrins: the role of an extracellular domain of integrin in intracellular signaling

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Summary

Integrins mediate cell adhesion and signal transduction at focal adhesions. Here we investigate the roles of integrin β subunits in the regulation of actin cytoskeletal structure and the activities of Rho and Rac. The overexpression of β3 integrin in Chinese hamster ovary cells enhances Rho activity and stress fiber formation, whereas the overexpression of β1 integrin increases Rac activity and lamellipodia formation. The overexpression of a mutant β1-3-1 integrin, in which the extracellular I-domain-like sequence of β1 integrin has been replaced with the corresponding sequence of β3 integrin, also enhances Rho activity and the formation of stress fibers. Our results demonstrate that β1 and β3 integrins differentially regulate the activities of Rho family GTPases and that the extracellular domains of integrin β subunits play a critical role in transducing the extracellular ligand-binding information into specific intracellular signaling events.

Key words: Integrin, Extracellular domain, Small GTPase, Signal transduction

Introduction

Integrins are a family of transmembrane adhesion receptors that mediate both the attachment of cells to the extracellular matrix (ECM) and the interaction between cells. Integrin-mediated adhesion regulates many cellular functions such as proliferation, differentiation, migration and apoptosis (Hynes 1992; Clark and Brugge 1995; Longhurst and Jennings 1998). Each integrin molecule is a heterodimer comprising α and β subunits. It has been suggested that different α and β subunits may regulate distinct intracellular signaling events through their specific domains. After binding with its ligand, the cytoplasmic domain of integrin β subunits interacts with focal adhesion proteins, causing the activation of focal adhesion kinase and Src (Solowska et al., 1989; Hayashi et al., 1990; Reszka et al., 1992). The transmembrane and extracellular domains of α subunits are important for the recruitment of signaling molecules such as Shc to integrins (Wary et al., 1996). The presence of an I-domain-like structure within the β1, β2 and β3 subunits is critical for ligand binding and regulation (Loftus et al., 1994; Bajt et al., 1995; Puzon-McLaughlin and Takada 1996; Tozer et al., 1996; Takada et al., 1997). When the sequence CTSEQNC (residues 187-193) in the extracellular I-domain-like structure of β1 integrin is replaced with the corresponding CYDMKTTC sequence of β3 integrin to create a mutant β1-3-1 integrin, the ligand

specificity is altered from that of β1 integrin to β3 integrin, and the integrin β1-3-1 recognizes the β3 integrin ligands fibrinogen and vitronectin (Takagi et al., 1997). However, the roles of different β subunits, especially their extracellular domains, in integrin-mediated signal transduction are not well understood.

Rho family GTPases (Rho, Rac and Cdc42), which function as binary switches that cycle between an active GTP-bound form and an inactive GDP-bound form, have distinct functions in regulating the actin-based cytoskeletal structure (Ridley and Hall 1992; Ridley et al., 1992; Van Aelst and D'Souza-Schorey 1997). Rho increases cell contractility, focal adhesions and actin stress fibers (Ridley and Hall 1992; Hotchin and Hall 1995), and Rac induces lamellipodia formation and membrane ruffles, as well as wavy, loose bundles of actin filaments in the periphery (Ridley and Hall 1992; Ridley et al., 1992; Rottner et al., 1999; Small et al., 1999). Recent studies have suggested that integrins can activate Cdc42, Rac and Rho (Hotchin and Hall 1995; Clark et al., 1998; Price et al., 1998; Ren et al., 1999). Fibroblasts plated on fibronectin exhibit an early activation of Cdc42 and Rac and a delayed activation of Rho (Price et al., 1998; Ren et al., 1999), suggesting that Cdc42/Rac and Rho may be regulated through different pathways. However, the molecular basis of the differential activation of Rho family GTPases is not clearly understood.

Here we showed that the overexpression of β3 integrin enhanced Rho activity and stress fiber formation, whereas the overexpression of β1 integrin increased Rac activity and lamellipodia formation. The overexpression of a mutant β1-3-1 integrin, in which the extracellular I-domain-like sequence of β1 integrin has been replaced by the corresponding sequence of β3 integrin, also enhanced Rho activity and stress fiber formation. These results suggest that the extracellular domains of β1 and β3 integrins play differential roles in transducing the extracellular stimuli to result in the specific regulation of Rac and Rho activities.

Materials and Methods

Cell culture and transfection

The Cell culture reagents were obtained from GIBCO BRL (Grand Island, New York, USA). Chinese hamster ovary (CHO) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, L-glutamine and non-essential amino acids. All cell cultures were maintained in a humidified 5% CO_2-95% air incubator at 37°C.

The methods of transfection of CHO cells to generate single transfectants expressing human β1, β3 and β1-3-1 integrins have been previously described (Takagi et al., 1997). The parent CHO cells did not express endogenous β3 integrin (Ylanne et al., 1993). Human β1, β3 or β1-3-1 integrin cDNA in pBJ-1 vector was co-transfected with pFneo into CHO cells. β1-3-1 integrin is a mutant in which the sequence CTSEQNC (residues 187-193) in the extracellular I-domain-like structure of β1 integrin is replaced with the corresponding CYDMKTTC sequence of β3 integrin. The transfected cells were then selected with G418. After selection, β1, β3 and β1-3-1 cells were cultured in the presence of G418 at 100 µg/ml in CHO cell culture medium. The expressed β integrins were detected at focal adhesions in each cell line (Takagi et al., 1997). The expression levels of human β1 integrin on β1 cells and β1-3-1 integrin on β1-3-1 cells were analyzed by flow cytometry using an anti-human β1 integrin antibody (AIIB2). β1 and β1-3-1 cell lines with equivalent expression level of β1 or β1-3-1 integrins were used in our experiments. The expression level of β3 integrin was not directly comparable with that of β1 integrin, owing to the different affinities of antibodies against β1 and β3 integrins.

For all experiments, culture dishes or slides were coated with fibronectin (2.5 µg/cm^2) or fibrinogen (5 µg/cm^2) for 2 hours, and the non-specific binding sites were blocked with 0.2% bovine serum albumin (BSA) (Calbiochem).

Actin staining and confocal microscopy

Cells were plated on coverslips that had been coated with either 2.5 µg/cm^2 fibronectin or 5.0 µg/cm^2 fibrinogen for 4 hours in serum-free DMEM. The plated cells were fixed in 3% paraformaldehyde in phosphate buffer saline (PBS) for 30 minutes and permeabilized with 0.5% Triton X-100 in PBS for 15 minutes. The specimens were then incubated with FITC-conjugated phalloidin (5 U/ml, Molecular Probes, Eugene, OR) for 1 hour, and the resulting actin staining was observed under a confocal microscopy system (MRC-1024, Bio-Rad, Hercules, CA).

Rho and Rac activities assay

The activities of Rho and Rac were measured by the recently developed affinity-precipitation assays on the basis of the specific interaction of the activated Rho family GTPases with their downstream effectors (Ren et al., 1999; del Pozo et al., 2000). Briefly, after the cells had been passed onto culture dishes coated with fibronectin for different durations in the absence of serum, the Rho-binding domain (RBD) from Rho effector Rhotekin and the p21-binding domain (PBD) from PAK were purified as GST fusion proteins. For the Rho activity assay, the cells were lysed with a high salt RIPA buffer (50 mM Tris-HCl, pH 7.2, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl$_2$, 10 µg/ml leupeptin, 1 mM orthovanadate and 1 mM PMSF) and centrifuged at 13,000 *g* at 4°C for 10 minutes. The cell lysates were incubated with 20 µg GST-RBD beads for 45 minutes at 4°C. The activated Rho was detected by immunoblotting using a monoclonal anti-RhoA antibody (Santa Cruz Biotechnology, Santa Cruz, CA). For Rac activity assay, the cells were lysed in a buffer containing 50 mM Tris-HCl pH 7.0, 0.5% NP-40, 500 mM NaCl, 5 mM MgCl$_2$, 5% glycerol, 10 µg/ml leupeptin, 1 mM orthovanadate and 1 mM PMSF. GST-PBD beads were used for affinity precipitation assays, and the activated Rac was detected by immunoblotting using an anti-Rac1 antibody (Transduction Laboratory). In the inhibition experiment, the PI 3-kinase inhibitor LY294002 (Sigma) was added to β1 cells in suspension for 30 minutes before seeding the cells on fibronectin.

PI 3-kinase activity assay

Cells were lysed in a buffer containing 50 mM HEPES, pH 7.5, 1% NP-40, 10% glycerol, 150 mM NaCl, 1.5 mM MgCl$_2$, 1.0 mM EDTA, 10 mM Na$_4$P$_2$O$_7$, 100 mM NaF, 1 mM PMSF, 1 mM Na$_3$VO$_4$, 10ug/ml leupeptin and 10 µg/ml aprotinin. The supernatant containing 500 µg protein was immunoprecipitated by using an anti-PI 3-kinase p85 polyclonal antibody (Santa Cruz Biotechnology). After washing, the beads were resuspended in 50 µl of the kinase buffer containing 0.2 mg/ml of phosphatidylinositol, 20 µM ATP, 20 µCi of [γ-^{32}P] ATP, and 20 mM MgCl$_2$. The sample was extracted once with 160 µl methanol-chloroform (1:1) and twice with 200 µl chloroform. The combined organic phase was washed once with 200 µl of 1M HCl-methanol (1:1). Phosphatidylinositol was recovered from the organic phase by evaporation, suspended in 15 µl chloroform and analyzed by thin layer chromatography (TLC) on a Silica gel 60 TLC plate (VWR, Willard, OH) (Whitman et al., 1988). The products were visualized by autoradiography. To detect the amounts of immunoprecipitated PI 3-kinase in each sample, 200 µg of protein lysates were immunoprecipitated by using an anti-PI 3-kinase p85 polyclonal antibody, and the immunoprecipitated complexes were separated by SDS-polyacrylamide gel electrophoresis (PAGE). The amount of PI 3-kinase p85 was detected by the anti-PI 3-kinase p85 polyclonal antibody.

JNK kinase activity assay

Cells were lysed in a kinase lysis buffer containing 25 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM EDTA, 50 mM NaF, 1 mM Na$_3$VO$_4$, 1 mM PMSF and 2 mM BGP. JNK was immunoprecipitated by using a polyclonal anti-JNK1 antibody (Santa Cruz Biotechnology) and protein A-Sepharose beads (Sigma). The immunocomplexes were incubated in 30 µl kinase buffer containing 25 mM HEPES, pH 7.4, 20 mM MgCl$_2$, 1 mM PMSF, 10 µg/ml leupeptin, 20 mM β-glycerophosphate, 1 mM Na$_3$VO$_4$ and 2 mM DTT, 2 µg of glutathione S-transferase (GST-c-Jun-1-79) fusion protein, 10 µCi [γ-^{32}P] ATP and 25 µM ATP at 30°C for 20 minutes. The reactions were stopped by the addition of a SDS sample buffer. The phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography.

Adhesion and detachment assays

The adhesion assay was performed as previously described (Takagi et al., 1997). Briefly, 96-well Immulon-2 microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with 2.5 µg/cm^2 fibronectin in 100 µl of PBS and incubated for 1 hour at 37°C. The remaining protein binding sites were blocked by incubating with 0.2% BSA for

1 hour at room temperature. Cells (2.5×10^4 cells/well) in 100 µl of DMEM were added to the wells and incubated at 37°C for 5 minutes. After removing the non-bound cells by rinsing the wells with the same buffer, the bound cells were quantified by measuring the endogenous phosphatase activity (Prater et al., 1991).

The detachment assay was used to detect the strength of cell-substrate adhesion, which was quantified by calculating the percentage of cells removed by well-defined shear flow. Cells were seeded on a fibronectin-coated (2.5 µg/cm²) glass slide and incubated at 37°C for 4 hours to allow the establishment of cell-substrate adhesion. The glass slide was then assembled into a parallel plate flow chamber (Hochmuth et al., 1973), and the cells were subjected to a dislodging shear stress of 250 dyn/cm² for 10 minutes. The numbers of cells before and after the application of the shear flow was counted in the same field of view from videotaped images. The results were expressed as the percentage of cells detached after the application of the shear flow.

Results

Changes in cell morphology that accompany cell spreading on fibronectin or fibrinogen

To test whether the β subunits regulate cell morphology differentially, we compared the cell morphology and actin organization among control CHO, β1, β3 and β1-3-1 cells. Serum (0.5%)-starved CHO, β1, β3 and β1-3-1 cells were plated on glass slides coated with fibronectin, which is a ligand for both β1 and β3 integrins, in serum-free DMEM. This serum-free condition avoids the potential crosstalk between integrin- and growth-factor-receptor-mediated signaling (Borges et al., 2000). Actin staining indicated the presence of lamellipodia and wavy, loose bundles of actin filaments in the periphery of CHO and β1 cells (Figs. 1A,B). Although β3 cells also showed lamellipodia, they differed from the CHO and β1 cells in having a larger amount of typical stress fibers (Fig. 1C). The morphology of β1-3-1 cells on fibronectin (Fig. 1D) was similar to that of β3, rather than being like β1 cells. As shown in Fig. 1G, the percentage of β3 and β1-3-1 cells with central typical stress fibers is significantly higher than those of CHO and β1 cells after 4 and 16 hours of adhesion on fibronectin. To prove that the change of extracellular I domain, rather than the expression level of human β integrins, was the reason for morphological differences between β1 and β1-3-1 cells, the expression level of human β integrin was measured by flow cytometry using an anti-human β1 integrin antibody. Mean fluorescence intensities for β1 and β1-3-1 cells were 61 and 59, respectively. Taken together, these results suggest that β3 integrin preferentially induces stress fiber formation and that the extracellular I-domain-like structures of β integrins, rather than their cytoplasmic domains, are responsible for the adhesion-induced morphological difference between β1 and β3 cells.

To further test the roles of β1 and β3 subunits in the formation of lamellipodia and stress fibers, CHO, β1, β3 and β1-3-1 cells were plated on coverslips coated with fibrinogen, which binds only to β3 but not to β1 integrin. As expected, CHO and β1 cells did not attach to the fibrinogen-coated coverslips because CHO cells did not express endogenous β3 subunit (Ylanne et al., 1993). β3 (Fig. 1E) and β1-3-1 (Fig. 1F) cells plated on fibrinogen had a comparable level of stress fiber formation to when these cells were plated on fibronectin.
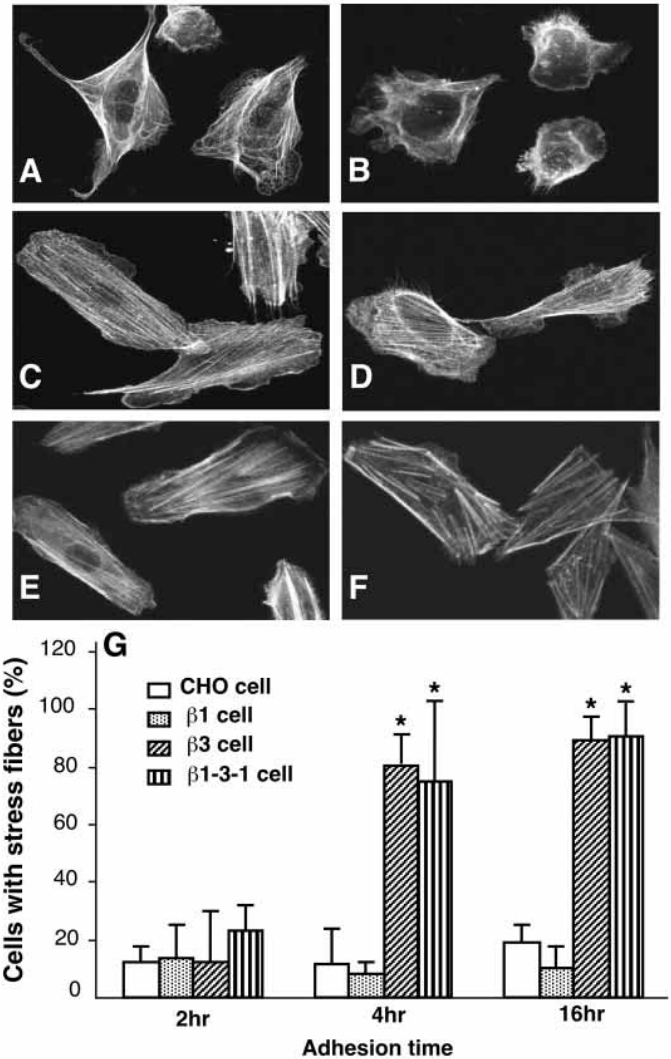

Fig. 1. Morphological changes in different types of CHO cells after plating on fibronectin and fibrinogen. CHO (A), β1 (B), β3 (C) and β1-3-1 (D) cells were plated for 4 hours on slides coated with fibronectin. β3 (E) and β1-3-1 (F) cells were also plated for 4 hours on slides coated with fibrinogen. Staining of actin was observed by confocal microscopy. In G, the percentage of cells with central stress fibers was determined in CHO, β1, β3 and β1-3-1 cells seeded on fibronectin-coated slides for 2, 4 and 16 hours. Error bars represent standard deviations from three separate experiments (*$P<0.05$ compared with CHO cells).

β1 and β3 integrins differentially regulate Rho and Rac activities to cause the different actin organization

To investigate whether β1 and β3 integrins differentially regulate Rho and Rac activities to cause the different actin organization, suspended CHO, β1, β3 and β1-3-1 cells were plated on fibronectin-coated culture dishes in serum-free DMEM for various lengths of time. RhoA activity was detected by RBD of Rhotekin and pull-down assays (Ren et al., 1999). As shown in Fig. 2, the level of RhoA activity in the CHO (Fig. 2A) and β1 (Fig. 2B) cells plated on fibronectin was lower than that in suspension, and this decrease remained significant for at least 4 hours ($P<0.05$). In contrast, the level of RhoA activity in β3 (Fig. 2C) and β1-3-1 cells (Fig. 2D), following an initial
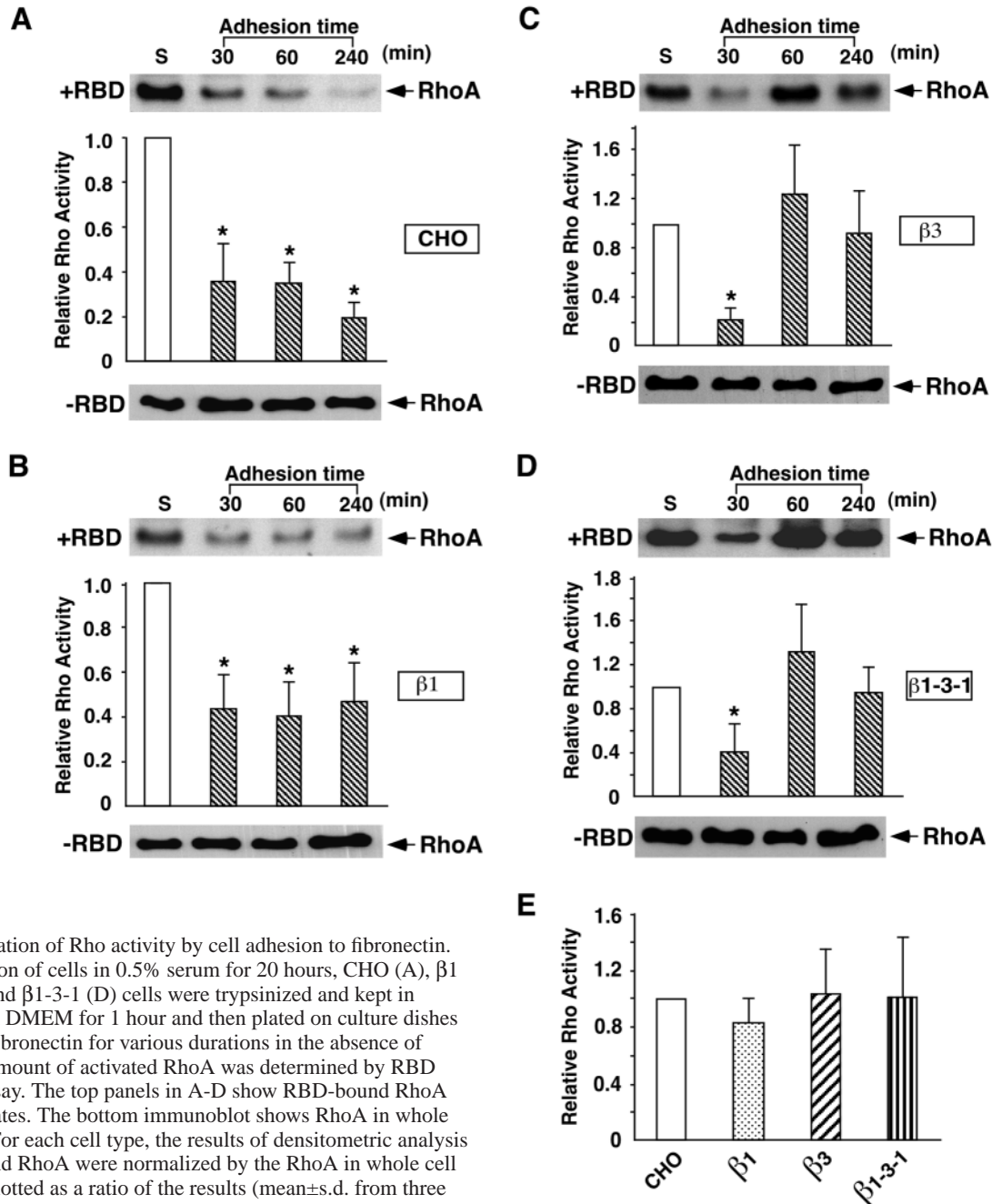
A

B

C

D

E

Fig. 2. Regulation of Rho activity by cell adhesion to fibronectin. After starvation of cells in 0.5% serum for 20 hours, CHO (A), β1 (B), β3 (C) and β1-3-1 (D) cells were trypsinized and kept in suspension in DMEM for 1 hour and then plated on culture dishes coated with fibronectin for various durations in the absence of serum. The amount of activated RhoA was determined by RBD pull down assay. The top panels in A-D show RBD-bound RhoA from cell lysates. The bottom immunoblot shows RhoA in whole cell lysates. For each cell type, the results of densitometric analysis of RBD-bound RhoA were normalized by the RhoA in whole cell lysates and plotted as a ratio of the results (mean±s.d. from three experiments). Asterisks indicate significant difference ($P<0.05$) from the ratio of 1. (E) RhoA activities of CHO, β1, β3 and β1-3-1 cells in suspension, normalized for whole cell lysates and expressed as a ratio to CHO cells (mean±s.d. from three experiments). There was no significant difference among the four groups of cells in suspension.

decrease in the early phase of adhesion, rose to become not significantly different from the level in suspension after 1 hour of adhesion ($P>0.30$). We did not detect any difference in Rho activity among CHO, β1, β3 and β1-3-1 cells in suspension (Fig. 2E). These results suggest that β3 integrin preferentially promoted the increase of Rho activity of adhered cells to the level seen in suspended cells and that the extracellular I-domain-like structure of β3 integrin is important for this effect on Rho activity.

To examine whether expression of β3 integrin and overexpression of β1 integrin affect Rac activity, we used PBD

and pull down assays (del Pozo et al., 2000). In contrast to Rho activity, the level of Rac1 activity (Fig. 3A) was higher in CHO and β1 cells after 4 hours of adhesion, suggesting that β1 integrin preferentially increases Rac activity. Again, there is no significant difference in Rac activity among different types of cells in suspension (Fig. 3B).

Overexpression of β1 integrin causes adhesion induction of PI 3-kinase and JNK activities

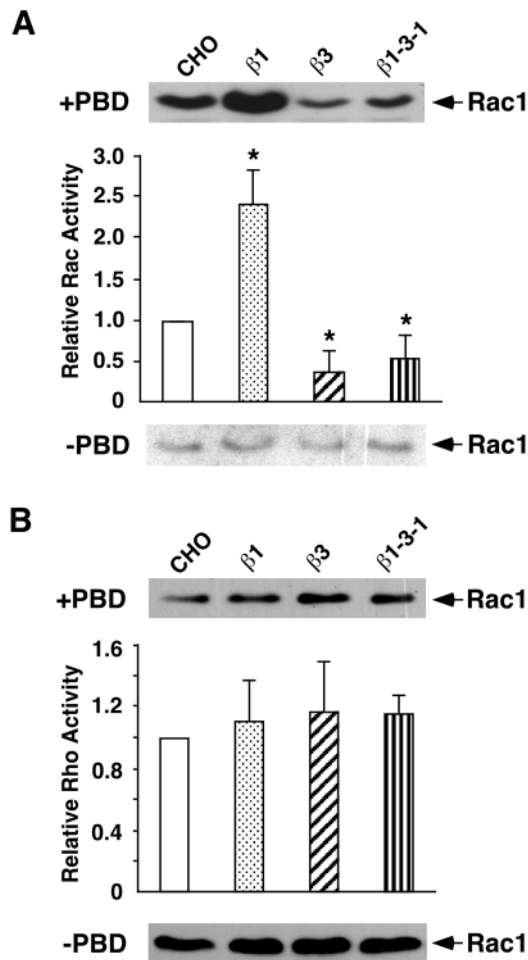Rac is known to be activated by PI 3-kinase and to stimulate a

A

B

Fig. 3. Regulation of Rac1 activity by cell adhesion to fibronectin. 0.5% serum-starved CHO, β1, β3 and β1-3-1 cells were detached and kept in suspension in DMEM for 1 hour and then either plated on fibronectin-coated dishes for 4 hours (A) or used as suspension controls (B). Rac1 activity was determined by a PBD assay followed by immunoblotting with an anti-Rac1 antibody. The bottom immunoblot shows Rac1 in whole cell lysates. The amount of activated Rac1 was normalized by the amount of Rac1 in whole cell lysates. The bar graphs are densitometric analyses of mean±s.d. from three separate experiments. The asterisk indicates significant differences ($P<0.05$) compared with CHO cells.

kinase cascade leading to the subsequent activation of JNK (Coso et al., 1995; Minden et al., 1995; Kiyono et al., 1999). To test whether overexpression of β1 integrin can enhance the activation of PI 3-kinase and JNK, we examined PI 3-kinase and JNK activities after plating cells on fibronectin for 4 hours. The activation of PI 3-kinase (Fig. 4A) was higher in β1 cells than that in CHO, β3 and β1-3-1 cells. To test the relationship between PI 3-kinase and Rac in this system, the specific PI 3-kinase inhibitor LY294002 was added to a β1 cell suspension 30 minutes before seeding the cells on fibronectin. Inhibition of PI 3-kinase with LY294002 blocked Rac activity in β1 cells (Fig. 4B), suggesting that PI 3-kinase is involved in the activation of Rac1 in this system. Consistent with the activation of PI 3-kinase and Rac1, JNK kinase activity in β1 cells was higher than that in CHO, β1 and β1-3-1 cells (Fig. 4C). These results suggest that β1 integrin activates JNK through the

activation of PI 3-kinase and Rac and that this signaling process is regulated by the extracellular I-domain-like structure of the β1 subunit.
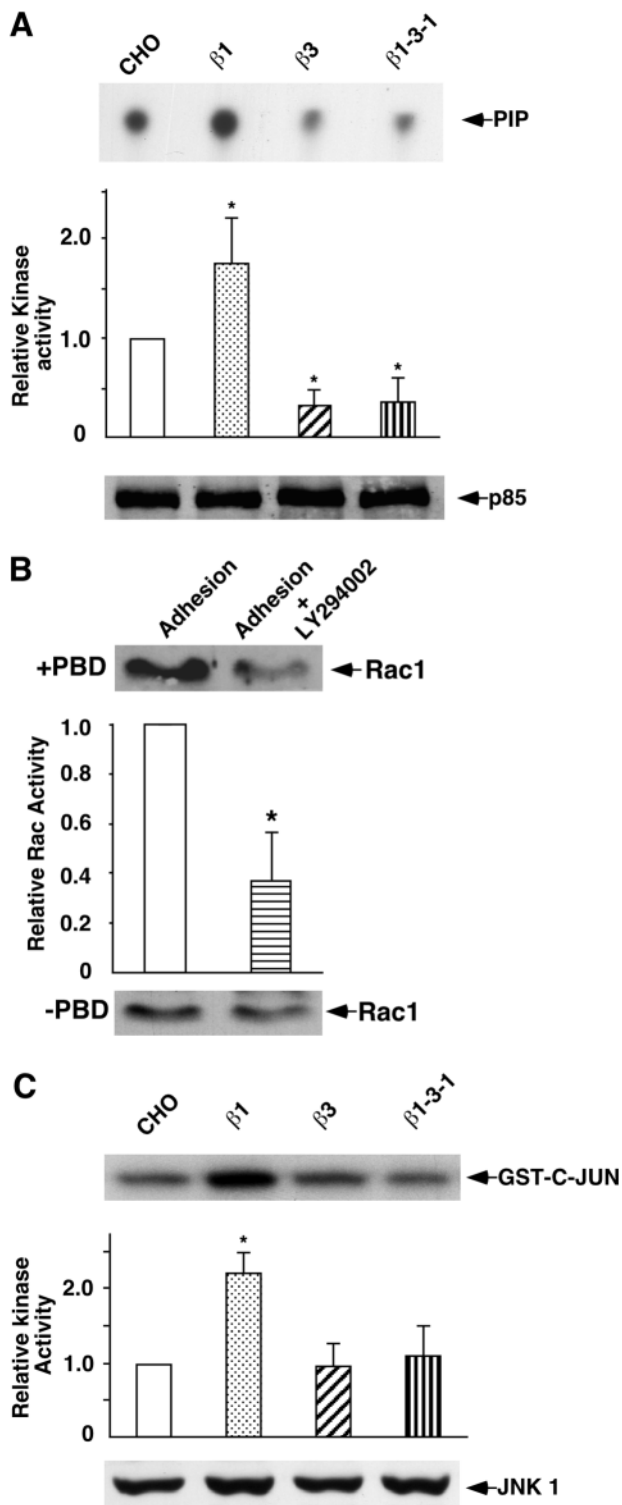
Adhesion and detachment assays

Two approaches were used to quantify cell adhesion. First, we studied the efficiency of cell attachment to a fibronectin-coated substrate. This adhesion assay provides a very gentle removal of non-adherent cells. We detected no significant differences in the number of bound cells in different cell lines (Fig. 5A). In the second approach, we applied a dislodging shear stress (250 dyn/cm^2) to the cells, which had established matured cell-substrate adhesion to test the strength of adhesion. As shown in Fig. 5B, we did not detect any significant difference in the number of detached cells in different cell lines. These results suggest that the different phenotypes of β1 and β3 cells were not caused by the differences in cell adhesion.

Discussion

Although it has been shown that cell adhesion on fibronectin mediates the activity of Rho GTPases (Hotchin and Hall 1995; Clark et al., 1998; Price et al., 1998; Ren et al., 1999), the integrins involved in this regulation have not been elucidated. Here we have shown that expression of β3 integrin in CHO cells enhanced Rho activity and stress fiber formation, whereas overexpression of β1 integrin increased Rac/JNK activity and lamellipodia formation (Figs 1-4). These results suggest, for the first time, that different integrins may differentially regulate Rho and Rac activities to induce different cellular responses. The activities of Rho and Rac in different types of cells in suspension were not significantly different, indicating that the differential regulation of Rho and Rac activities is adhesion dependent. The different phenotypes of β1 and β3 cells cannot be explained by differences in cell adhesion, since we did not detect any significant difference in cell adhesion between these cells by using adhesion and detachment assays (Fig. 5). Our experiments were performed by using CHO cells, which normally express β1 but not β3 integrin (Ylanne et al., 1993; Takagi et al., 1997). The transfected β subunits were associated with the various endogenous α subunits in CHO cells (Y.T., unpublished). Since different cell types may have different combinations of integrins and signaling molecules, it is not clear whether our conclusion can be generalized to other cell types. Interestingly, we found that endothelial cells on collagen had more lamellipodia than those on fibrinogen (H.M. and S.C., unpublished), which is consistent with our observation in CHO cells.

The I-domain-like structure within the β1, β2 and β3 subunits has been reported to have components critical for ligand binding and regulation (Loftus et al., 1994; Bajt et al., 1995; Puzon-McLaughlin and Takada 1996; Tozer et al., 1996; Takada et al., 1997). The CHO cells that express human β1-3-1 integrin provided a cell system that was ideally suited to evaluate the role of the extracellular I-domain-like structure of the integrin β subunits in integrin-mediated changes in cell morphology and Rho family GTPase activity. Expression of a mutant β1-3-1 integrin enhanced Rho activity and stress fibers so that there was higher activity than there was in β1 cells, that is, this resulted in the same phenotype as β3 rather than being

A

B

C

Fig. 4. Regulation of PI 3-kinase and JNK activities by cell adhesion to fibronectin, and the effect of PI 3-kinase inhibitor on the adhesion induction of Rac1 activation. 0.5% serum-starved CHO, β1, β3 and β1-3-1 cells were detached and kept in suspension in DMEM for 1 hour and then plated on fibronectin-coated dishes for 4 hours. In A, cell lysates from the various samples following adhesion to fibronectin were immunoprecipitated with anti-PI 3-kinase p85 antibody followed by the PI 3-kinase activity assay. Shown at the bottom is the amount of PI 3-kinase p85 immunoprecipitated from 200 μg cell lysates, indicating that comparable amounts of PI 3-kinase p85 were immunoprecipitated in these samples. (B) The effect of a PI 3-kinase inhibitor, LY294002, on adhesion induction of Rac1 activity is shown in the top panel. The bottom immunoblot shows Rac1 in whole cell lysates. The amount of activated Rac1 was normalized by the amount of Rac1 in whole cell lysates. (C) The top panel shows JNK kinase activity using GST-c-Jun as the substrate, and the bottom panel is the immunoblot using anti-JNK1, which indicates equal loading. The bar graphs in A-C are the densitometric analyses, representing the mean±s.d. of three separate experiments. The asterisks in A and C indicate significant differences (*P*<0.05) compared with CHO cells, and the asterisk in B indicates a significant difference (*P*<0.05) compared with cells without the PI 3-kinase inhibitor.
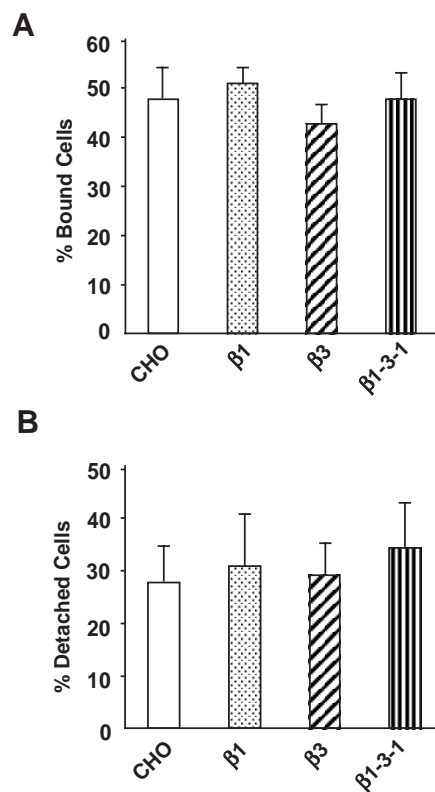
A

B

Fig. 5. Attachment and detachment of cells on fibronectin. (A) Cells were seeded on fibronectin-coated wells and the percentage of bound cells was quantified by the adhesion assay described in the Materials and Methods. (B) Cells were seeded on fibronectin-coated slides and then subjected to a dislodging shear stress of 250 dyn/cm². The percentage of detached cells was quantified. Bars represent mean±s.d. of three separate experiments.

like β1 cells. Since both the expression level of β1 and β1-3-1 integrins and the adhesion force of β1 and β1-3-1 cells were similar, our findings indicate that the extracellullar I-domain-like structures of the integrin β subunits play an important role in β1- and β3-integrin-mediated increases of Rho and Rac activities. The molecular mechanisms involved in the differential regulation of Rho and Rac by β1 and β3 integrins are not known. One possibility is that different ligand binding

on the extracellular domains of β subunits may change the conformation of the integrins, thus affecting the recruitment of signaling molecules at focal adhesions and the anchorage of the cytoskeleton.

There are many possible mediators for the differential modulation of Rho and Rac activities by integrins. Tyrosine phosphorylation has been shown to regulate Rho and Rac activities (for a review, see Kjoller and Hall 1999), but we did not detect significant difference in the level of tyrosine phosphorylation in CHO, β1, β3 and β1-3-1 cells (H.M. and S.C., unpublished). Our data indicate that PI 3-kinase mediates the enhancement of Rac activity induced by the overexpression of β1 integrin (Fig. 4). PI 3-kinase could stimulate Rac activity through its lipid product phosphatidylinositol 3,4,5-trisphosphate, which binds to the PH domain of guanine nucleotide exchange factors (GEFs), such as Tiam1, Sos and Vav (Rameh et al., 1997; Han et al., 1998). It is likely that the differential regulation of Rho and Rac by integrins is mediated by the differential regulation of GEFs, GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs). Indeed, some of the GEFs and GAPs are specific regulators of particular Rho family members. For example, GEF Lbc and GAP p122 specifically regulate Rho activity (Homma and Emori 1995; Zheng et al., 1995), whereas GEF Tiam and GAP chemerin specifically regulate Rac activity (Diekmann et al., 1991; Habets et al., 1994). It is possible that different integrin β subunits modulate the functions of one or more GEFs, GAPs and GDIs, thus changing the overall balance of these regulators. Such differential regulation may go through different signaling molecules and their combinations and/or different through feedback regulation at focal adhesions.

The coordinated regulation of Rho GTPases is complicated. In fibroblasts, Cdc42 activates Rac, which in turn activates Rho to regulate the actin cytoskeleton (Nobes and Hall 1995), suggesting a hierarchical cascade for Rho GTPases. However, the differential regulation of Rho and Rac has been shown under some circumstances. For example, the growth of microtubules can lead to the activation of Rac (Waterman-Storer et al., 1999), whereas disruption of microtubules leads to the activation of Rho (Ren et al., 1999). Downstream in the activation pathway, Rac can counter the effect of Rho by decreasing the phosphorylation of myosin light chain (Sanders et al., 1999). Our data indicate that β1 integrin increases Rac activity, whereas β3 integrin enhances Rho activity. Since Rac and Rho regulate different downstream effectors in signal transduction and actin cytoskeleton organization, the signals from β1 and β3 integrins may complement each other in the regulation of actin filament assembly and signaling events.

In summary, we have shown that β1 and β3 integrins differentially regulate Rac and Rho activities and actin organization. Therefore, β1 and β3 integrins could play differential as well as coordinated roles in modulating cell functions such as migration and proliferation.

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